SPECIFICATION

HEAT- OR SINGLET OXYGEN-GENERATING AGENTS AND CANCER TREATMENT COMPOSITIONS COMPRISING ORGANIC PEROXIDE OR CHEMILUMINESCENT COMPOUND

Technical Field

[0001] The present invention relates to a heat- or singlet oxygen-generating agent comprising an organic peroxide or a chemiluminescent compound and to a pharmaceutical composition which, entirely unlike conventional ones, uses heat or singlet oxygen to exhibit anticancer effect.

Background Art

[0002] In general, conventional cancer treatments include a method using anticancer drug that is an alkylating agent and a method for generating singlet oxygen using light. However, these methods have such drawbacks that cancer cells easily acquire resistance to the drug and that there are serious side effects. Besides, thermotherapy which has been used conventionally merely warms a patient at a hot spring and is not expected to act directly on cancer cells.

Disclosure of the Invention

Problems to be Solved by the Invention

[0003] Therefore, an object of the present invention is to provide a cancer treatment drug which, compared to conventional anticancer drugs, hardly develops above-mentioned side effects and is hardly tolerated, and is capable of reducing such burden on a patient.

Means for Solving the Problems

[0004] The present invention is (1) a heat- and/or singlet oxygen-generating agent comprising an organic peroxide or a chemiluminescent compound.

[0005] The present invention is (2) the generating agent according to above-mentioned (1), wherein the generating agent is used for anticancer or inducing sudden death of cells.

[0006] The present invention is (3) the generating agent according to above-mentioned (1) or (2), wherein the generating agent generates heat and/or singlet oxygen under the environment of a site where cancer cells are present.

[0007] The present invention is (4) the generating agent according to any of above-mentioned (1) - (3), wherein the incorporation into cells is accelerated.

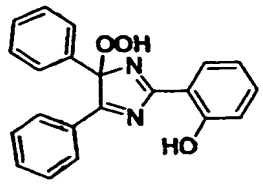
[0008] The present invention is (5) the generating agent according to any of above-mentioned (1) - (4), wherein the organic peroxide is a peroxide of an imidazole derivative.

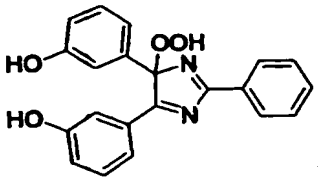
[0009] The present invention is (6) the generating agent according to any of above-mentioned (1) - (4), wherein the chemiluminescent compound is a dioxetane compound.

[0010] The present invention is (7) a pharmaceutical composition for cancer treatment, comprising an organic peroxide or chemiluminescent compound generating heat and/or singlet oxygen.

[0011] The present invention is (8) a pharmaceutical composition for inducing sudden death of cells, comprising an organic peroxide or a chemiluminescent compound generating heat and/or singlet oxygen.

[0012] The present invention is (9) a compound represented by


OOH
N
N
HO


HO
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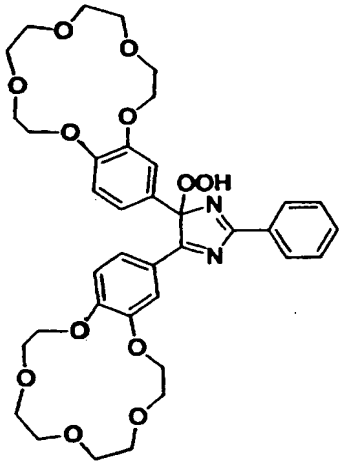
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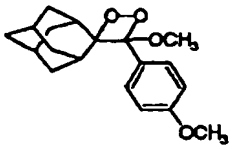
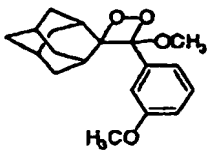
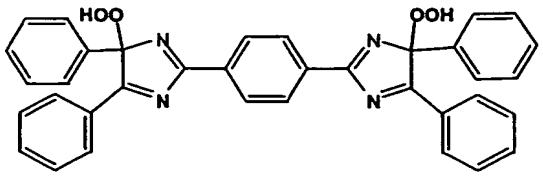
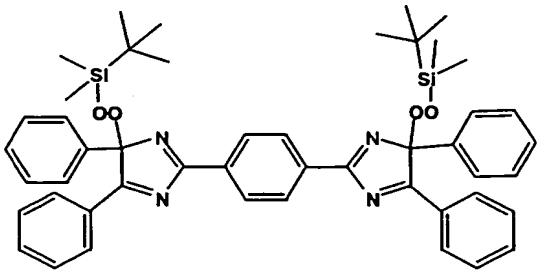
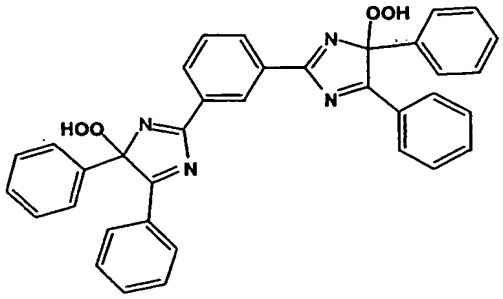

OCH3
OCH3

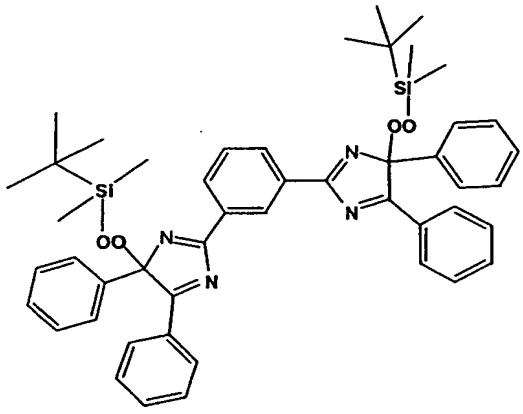

OCH3
H3CO







or



Best Mode for Carrying Out the Invention

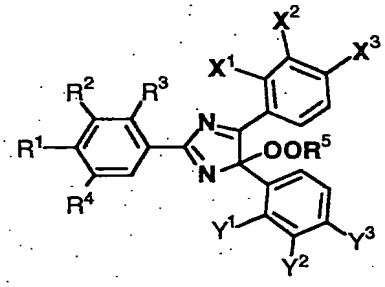
[0013] The organic peroxide according to the present invention includes, for example, a hydroperoxide, a percarboxylic acid, a dialkyl peroxide, a diacyl peroxide, an ester peroxide, a cyclic peroxide, an organic metal

peroxide, a peroxide of an imidazole derivative, and is preferably a peroxide of an imidazole derivative.

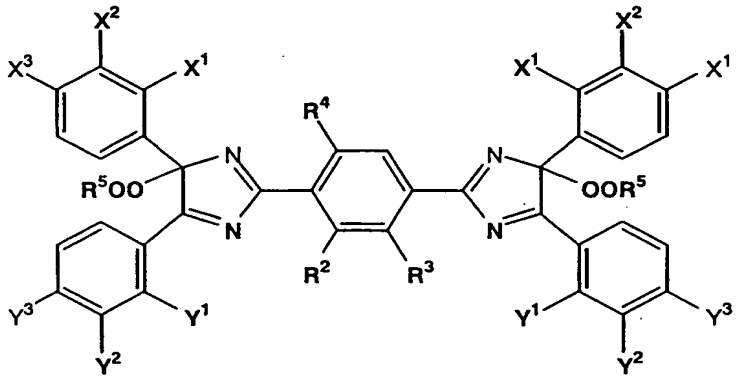
[0014] For the peroxide of imidazole derivative, for example, 4-hydroperoxides and 4-silyl peroxides of an imidazole are particularly preferred, and the endoperoxide is also included.

[0015] As the peroxide of imidazole derivative, for example, the following compounds are mentioned:

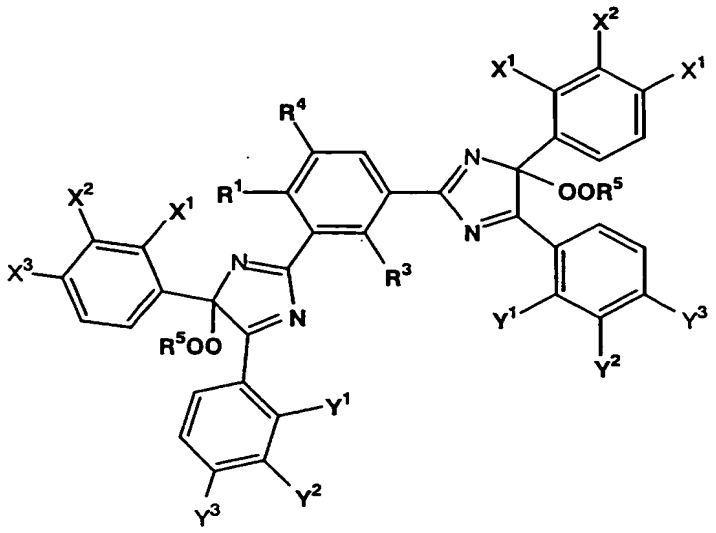
General formula 1:



General formula 2:



General formula 3:



[0016] In general formulae 1, 2 and 3, R^1 to R^4 denote a substituent group or an atomic group, and they are not limited particularly as long as they improve functions of the peroxide of imidazole as an anticancer agent. R^1 to R^4 independently represent a hydrogen atom or an appropriate substituent group. As the substituent group as R^1 to R^4, for example, a lower alkyl substituted amino group such as a primary amino group, a methylamino group, and a dimethylamino group, a halogen group such as a fluoro group, a chloro group, a bromine group, and an iodine group, a hydroxy group, a carboxyl group, a cyano group, a nitro group, and a formyl group are mentioned, and furthermore, in any of above-mentioned substituent group, one or more hydrogen atoms thereof may be further substituted by above-mentioned other substituent groups. The peroxide of an imidazole in which R^1 and/or R^2 is a hydroxyl group is preferred. In the peroxide of an imidazole in which R^1 is a lower alkyl substituted amino group, the alkyl group in the alkyl amino group may be bound to an adjacent carbon atom each other such as a carbon atom to which R^2 and/or R^4 is bound, to form a ring structure such as a piperidine ring and a julolidine ring.

Besides, R^1 to R^4 may be same or different each other and a heterocyclic group or an aromatic ring group of monocyclic or condensed polycyclic type, and the heterocyclic group or the aromatic ring group may have one or more substituent groups. As the heterocyclic group of R^1 to R^4, for example, an imidazoline ring, an imidazole ring, an oxazoline group, an oxazole ring, an isoxazole ring, a thiazoline ring, a thiazole ring, an isothiazole ring, a pyrrole ring, and a furan ring are mentioned, and as the aromatic ring group, for example, a benzene ring, a naphthalene ring, and an anthracene ring are mentioned.

[0017] In general formulae 1, 2 and 3, R^5 represents a substituent group or an atomic group, and they are not limited particularly as long as they improve functions of the peroxide of imidazole as an anticancer agent. For example, a functional group removable under a hydrolysis condition such as a hydrogen atom, a trimethylsilyl group, a dimethyl t-butylsilyl group, a triisopropyl silyl group, and an acyl group are mentioned.

[0018] In general formulae 1, 2 and 3, X^1, X^2 and X^3 represent a substituent group or an atomic group, and they are not limited particularly as long as they improve functions of the peroxides of imidazole as an anticancer agent. For example, a lower alkyl substituted amino group such as a primary amino group, a methylamino group and a dimethylamino group, a halogen group such as a fluoro group, a chloro group, a bromine group, and an iodine group, a hydroxy group, a carboxy group, a cyano group, and a nitro group are mentioned, and furthermore, in any of above-mentioned substituent groups, one or more hydrogen atoms thereof may be further substituted by above-mentioned other substituent groups.

[0019] In general formulae 1, 2 and 3, Y^1, Y^2 and Y^3 represent a substituent group or an atomic group, and they are not limited particularly as long as they improve functions of the peroxide of imidazole as an anticancer

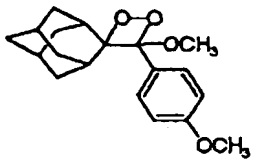
agent. For example, a lower alkyl substituted amino group such as a primary amino group, a methylamino group, and a dimethylamino group, a halogen group such as a fluoro group, a chloro group, a bromine group, and an iodine group, a hydroxy group, a carboxy group, a cyano group, and a nitro group are mentioned, and furthermore, in any of above-mentioned substituent groups, one or more hydrogen atoms thereof may be further substituted by above-mentioned other substituent groups.

[0020] Compounds of general formulae 1, 2 and 3 according to the present invention generate heat and singlet oxygen.

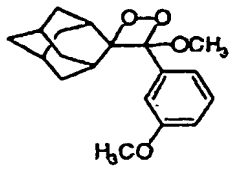
[0021] As the chemiluminescent compound according to the present invention, compounds of firefly luciferin, Vargula luciferin, luminol, acridine, lucigenin and dioxetane compounds are mentioned, and the dioxetane compound is preferred.

[0022] As the dioxetane compound, for example, compounds of tetraalkyl dioxetane, dioxetanone, and dioxetadione are mentioned.

[0023] As the preferred dioxetane compound, 3-(2'-spiroadamantane)-4-methoxy-4-(4"-methoxy) phenyl-1,2-dioxetane represented by



and 3-(2'-spiroadamantane)-4-methoxy-4-(3"-methoxy) phenyl-1,2- dioxetane represented by



are mentioned.

[0024] The chemiluminescent compound according to the present invention generates heat.

[0025] In the present invention, a heat generating agent

or an anticancer agent comprising an imidazole peroxide derivative or dioxetane compound generates a reaction heat of approximately 20 Kcal/mol to 90 Kcal/mol. Meanwhile, singlet oxygen is generated at a yield of approximately upto 50%. The MTT antitumor sensitivity test in which a cell line of large intestine tumor was incubated with an imidazole peroxide derivative for 48 hours revealed that the derivative exhibited sharply effects at 50 - 100 $\mu M/cm^2$ and showed high level of antitumor performance same as a commercially available MMC to give a survival rate of 11%.

[0026] Compounds formed from the 4-hydroperoxide and the 4-silyl peroxide of imidazoles are corresponding amidines, imidazoles, and, in some cases, singlet oxygen having cell activity, which are normally considered to be nontoxic. Further, compounds formed from dioxetanes are corresponding ketones. Therefore, the heat generating agent and the pharmaceutical composition according to the present invention can minimize side effects caused by deactivation or death of normal cells. Moreover, these have the feature of inducing sudden death of cancer cells. The cancers, which are curable by the present invention, include, but are not particularly limited to, liver cancer, lung cancer, stomach cancer, large intestine cancer, skin cancer and uterine cancer.

[0027] In the present invention, in order to deliver the heat generating agent or the pharmaceutical composition to cancer cells, they are applied to an affected area or normally injected. In addition, for example, a medical catheter is inserted from an inguinal region or the like, passed through blood vessels to target a cancer site, and then the heat generating agent or the pharmaceutical composition in solution is transferred through the catheter. Alternatively, they may be directly delivered to the lesion using a syringe, and in this case, they are preferably delivered in such a way that the cancer cells

may be killed as quickly as possible.

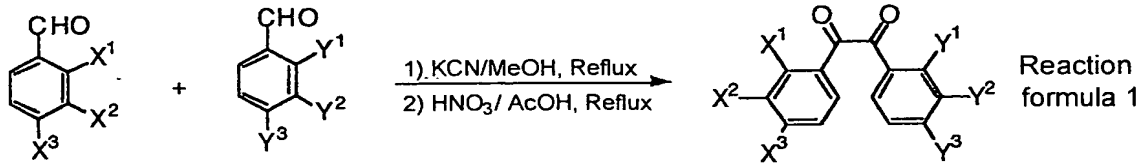
[0028] In the present invention, products formed by degradation of the organic peroxide or the chemiluminescent compound preferably have easily metabolizable structures. The peroxide of imidazole derivative or its endoperoxide or the dioxetane compound in the present invention is preferable to use, because it produces the corresponding imidazole, amidine or ketone which is diffused in a living body to give little influence on normal cells.

[0029] Further, in order to enhance degradation of the organic peroxide such as the peroxide of imidazole derivative or the chemiluminescent compound such as the dioxetane compound, an aqueous solution of KOH or NaOH, an organic base amine or an inorganic base containing F^- can be injected to an affected portion to accelerate the reaction.

[0030] It is preferable that the peroxide of imidazole, its endoperoxide or the dioxetane compound is decomposed to give a product which has a easily metabolizable structure, and that a small amount of the peroxide is administered.

[0031] The peroxide of an imidazole derivative represented by general formula (1) in the present invention can be synthesized by the method of, for example, reaction formula 1, reaction formula 2 and reaction formula 3 (and reaction formula 4) shown below.

[0032] Synthesis method 1 (Synthesis of benzils)

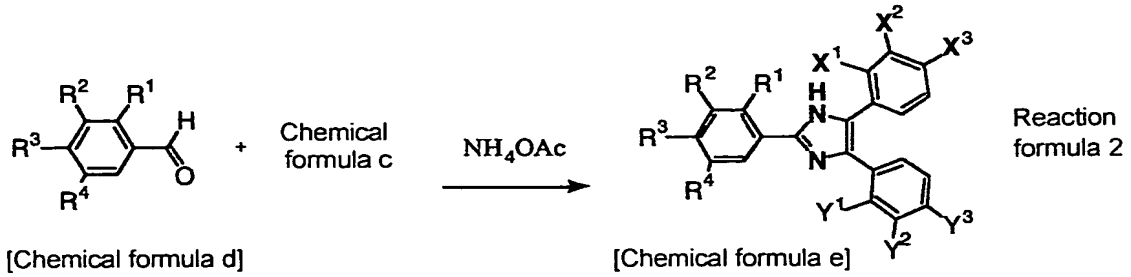


[Chemical formula a]

[Chemical formula b]

[Chemical formula c]

[0033] Synthesis method 2 (Synthesis of imidazoles)



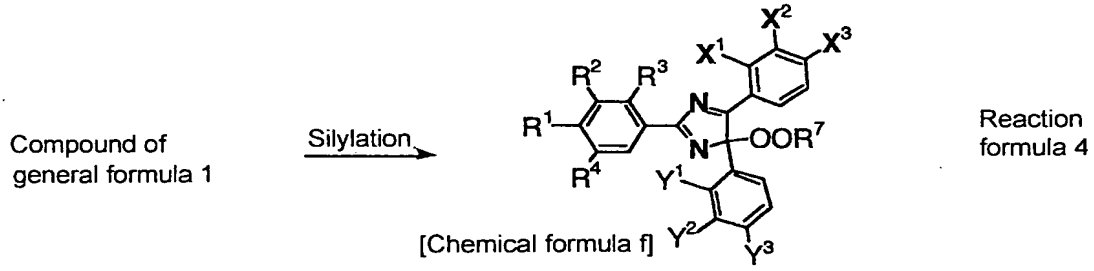
[Chemical formula d] [Chemical formula e]

[0034] Synthesis method 3 (Synthesis of peroxides)

Reaction is carried out in accordance with reaction formula (3) by the method of White et al. to synthesize the peroxide of an imidazole derivative represented by general formula (1), including the peroxide wherein X and Y are, in general, different substituent groups.

[0035] Synthesis method 4 (Synthesis of alkylsilyl derivatives)

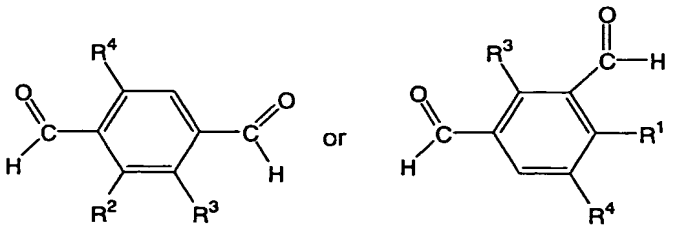
Trialkylsilyl derivatives of the peroxide represented by general formula (1) can be synthesized by reaction in accordance with reaction formula 4.



[Chemical formula f]

(In the chemical formula, R^7 denotes a trialkylsilyl group, and the alkyl group is a straight chain or branched chain alkyl group of C_1 - C_6, particularly C_1 - C_4.)

[0036] Imidazole derivatives represented by general formula (2) or (3) in the present invention and the silylation product thereof can be synthesized using the compound represented by

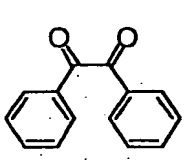


in the place of the compound of [Chemical formula d] in the synthesis method 2 of the imidazole derivative represented by general formula (1).

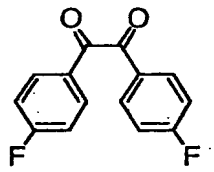
EXAMPLES

[0037] The present invention will be explained hereafter by, but is not limited to, Examples and Test examples.

[0038] Example 1



[Chemical formula g]



[Chemical formula h]

Benzils to use as the raw materials were synthesized according to reaction formula 1 by benzoin condensation of corresponding benzaldehyde followed by nitric acid oxidation. Contrastive [Chemical formula g] was synthesized by the method of Davidson et al. (Davidson, D.; Weiss, M.; Jelling. J. Org. Chem. 1937, 2, 319) and [Chemical formula h] was synthesized by the method of Luts et al. (Luts, R. E.; Murphey, R.S.J. Am. Chem. Soc, 1949, 71, 478).

[0039] Example 2

Imidazole derivatives were synthesized in accordance with Reaction formula 2 using the method of Davidson et al. (Davidson, D.; Weiss, M.; Jelling. J. Org. Chem. 1937, 2, 319). [Chemical formula g] or [Chemical formula h] was refluxed with one equivalent or a slightly excessive amount of the corresponding substituted

benzaldehyde and 10 equivalents of ammonium acetate in acetic acid for 4 - 5 hours, processed by a conventional method, and purified by recrystallization to get the corresponding imidazoles (compounds shown below) at their reasonable yields (50 - 80%). Their respective spectral data and the like are shown below.

2,4,5-Triphenyl imidazole(D. Davidson, M.Weiss, and M. Jelling, J. Org. Chem., 1937, 2, 319): colorless needle; mp 282.5-283°C; IR(KBr) 1613(C=N) cm^{-1}; ^1H NMR(500 MHz, $CDCl_3$) δ7.13-7.60(m, 13H), 8.08(d, J=8.3 Hz, 2H), 12.7(s, 1H); UV-vis λ_{max}(EtOH) 303(log ε 4.42)nm; MS(FAB) m/z 297(M^++1); HRMS(FAB) Calcd for $C_{21}H_{17}N_2$ 297.1392, Found 297.1424; Anal. Calcd for $C_{21}H_{16}N_2$: C, 85.11; H, 5.44; N, 9.45. Found: C,85.08; H, 5.48; N, 9.43.

4,5-Bis(4-fluorophenyl)-2-(4-dimethylaminophenyl)imidazole: colorless needle; mp 232-233°C; IR(KBr) 1620(C=N)cm^{-1}; ^1H NMR(500 MHz, $CDCl_3$) δ3.01(s, 6H), 6.71(br s, 2H), 7.00(br s, 4H), 7.45(br s, 4H), 7.83(br s, 2H); UV-vis λ_{max}(CH_2Cl_2) 230(log ε 4.1), 323(4.5) nm; Anal. Calcd for $C_{23}H_{19}F_2N_3$: C, 73.58; H, 5.10; N, 11.19. Found

2-(4-Hydroxyphenyl)-4,5-diphenylimidazole [A.H.Cook, D. G. Jones; J. Che. Soc., 278(1941)]: colorless needle; mp 273-275°C; ^1H NMR(200 MHz, DMSO-d_6)δ 6.84(d, J=8.4 Hz, 2H), 7.20-7.59(m, 10H), 7.88(d, J=8.4 Hz, 2H), 9.70(s, 1H), 12.4(s, 1H); IR(KBr) ν_{max} 3162(O-H), 1613(C=N), 1493, 1466, 1396, 1224, 1180, 839, 766, 739, 698 cm^{-1} ; UV-vis(EtOH); λ_{max} 221(log ε=4.27), 298(4.43) nm; HRMS(FAB) Calcd for $C_{21}H_{17}N_2O$ 313.1341($M+H^+$), Found 313.1341; Anal. Calcd for $C_{21}H_{16}N_2O\cdot H_2O$: C, 76.34; H, 5.49; N, 8.48. Found: C, 76.46; H, 5.69; N 8.23.

2-(3-Hydroxyphenyl)-4,5-diphenylimidazole(F. R. Japp, H. H. Robinson; Chem. Ber., 15, 1269(1882): colorless plate; mp 273-275°C; ^1H NMR(200 MHz, DMSO-d_6) δ 6.77(d, J=7.2 Hz, 1H), 7.16-7.58(m, 13H), 9.55(s, 1H), 12.6(s, 1H); IR(KBr) ν_{max} 3380(O-H), 1593(C=N), 1483, 1448, 1400, 1352, 1230, 1193,

791, 764, 729, 696 cm^{-1}; UV-vis(EtOH) λ_{max} 222(log ε =4.43), 304(4.43) nm; HRMS(FAB) Calcd for $C_{21}H_{17}N_2O$ 313.1341($M+H^+$), Found 313.1342; Anal. Calcd for $C_{21}H_{16}N_2O$: C, 80.75; H, 5.16; N, 8.97. Found: C, 80.65; H, 5.19; N, 8.92.

2-(4-Aminophenyl)-4,5-diphenylimidazole(Kallel&Co. Akt.-Ges. Ger., 1956, 950, 618): colorless needle; mp 253-256°C (literature value 180°C); IR(KBr)3360(N-H), 1613(C=N)cm^{-1}; ^1H NMR(500MHz, $CDCl_3$)δ5.23(s,2H), 6.48(d, J=8.5 Hz, 2H), 7.25(m, 10H), 7.60(d,J=8.5 Hz, 2H), 12.4(br s, 1H); UV-vis λ_{max}(EtOH)309(log ε 450) nm; MS(FAB) m/z 312(M^++1; 100%); HRMS(FAB) Calcd for $C_{21}H_{18}N_3$ 312.1501, Found 312.1483; Anal. Calcd for $C_{21}H_{17}N_3 \cdot 2/3H_2O$: C,77.99; H,5.71; N,12.99. Found: C,77.77; H,5.73; N,13.01.

2-(4-Nitrophenyl)-4,5-diphenylimidazole: yellow needle; mp 255-257°C; IR(KBr) 1603(C=N), 1518(NO_2), 1342(NO_2)cm^{-1}; ^1H NMR(500MHz, $CDCl_3$)δ7.35(m,10H), 8.09(d,J=8.4Hz, 2H), 8.32(d,J =8.4Hz, 2H), 9.60(s,1H); UV-vis λ_{max}(EtOH)222(log ε 4.28), 255(420), 386(4.29) nm; MS(FAB) m/z 342(M^++1; 100%); Anal. Calcd for $C_{21}H_{15}N_3O_2$: C,73.89; H,4.43; N,12.31. Found: C,73.87; H,4.48; N,12.24.

2-(4-Formylphenyl)-4,5-diphenylimidazole(B. Radziszewskii, Ber., 1877, 10, 70): yellow needle; mp244-245.5°C; IR(KBr) 2970(C-H), 1698(C=0), 1607(C=N), 837,766,696 cm^{-1}; ^1H NMR(200MHz, $CDCl_3$) δ7.19-7.68(m,10H), 7.97(d, J=8.4Hz, 2H), 8.10(d, J=8.4Hz, 2H), 10.05(s, 1H); UV-vis λ_{max}(EtOH) 243(log ε 4.14), 301(3.94), 359(4.24) nm; MS(FAB) m/z 325(M^++1); HRMS(FAB) $C_{22}H_{16}N_2O$ 325.1341, Found 325.1311; Calcd for Anal. Calcd for $C_{22}H_{16}N_2O$: C,81.46; H,497; N,8.64. Found: C,81.21; H,5.02; N,8.58.

2-(2',4',6'-Trimethylphenyl)-4,5-diphenylimidazole [G. R. Coraor, L.A. Cescon, R.Dessauer, E.F.Silversmith and E.J.Urban J. Org. Chem., 1971, 36(16), 2262-2267] :colorless needle; mp 242-243°C; IR(KBr) 2922(C-H), 1605(C=N)cm^{-1}; ^1H NMR(500MHz, $CDCl_3$)δ2.23(s, 6H), 2.32(s,3H), 6.93(s, 2H), 7.32(br s, 6H), 7.47(br s, 2H), 7.69(br s, 2H), 8.81(br s, 1H); UV-vis λ_{max}(EtOH) 222(log ε 439),

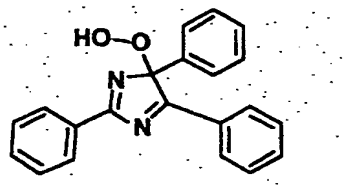
284(4.17) nm; MS(FAB)m/z 339(M^++1); HRMS(FAB) Calcd for $C_{24}H_{23}N_2$ 339.1861, Found 339.1860; Anal. Calcd for $C_{24}H_{23}N_2$ 1/2H_2O: C,82.96; H,6.67; N,8.06. Found: C,83.07; H,6.86; N,7.93.

[0040] Example 3

The peroxide of an imidazole derivative was synthesized according to reaction formula (3) by the method of White et al. (EH. White and M.J.C. Harding, Photochem. Photobiol., 1965, 4, 1129 - 1155).

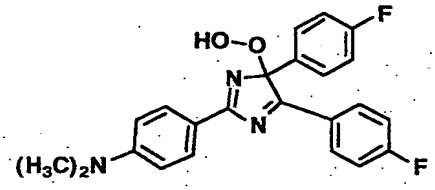
[0041] Various imidazole derivatives obtained in Example 2 were dissolved into dichloromethane at −78°C, added with a few drops of methylene blue as a sensitizer, and irradiated with artificial daylight while blowing oxygen for 4 - 6 hours. Upon completion of the reaction, the reaction mixtures were immediately added with alcohol, and subjected to evaporation of dichloromethane at a low-temperature (15°C or lower) to separate. Crystals thus obtained were washed with alcohol to get the peroxides (Chemical formula A - Chemical formula H, and Chemical formula J - Chemical formula L) shown below at high purities and high yields. Further, the peroxide similarly obtained was subjected to silylation by the following method to get silylated peroxide (Chemical formula I).

(Silylation method): The method of Corey et al. was used for silylation of peroxides (E.J. Corey and A. Venkateswaru, J. Am. Chem. Soc., 1972, 94, 6190-6191. G.R. Clark, M.M. Nikaido, C.K. Fair and J. Lin, J. Org. Chem., 1985, 50, 1994-1996). Namely, the peroxide was added with 5 equivalents of tert-butyldimethylsilyl chloride and a catalytic amount of pyridine, and subjected to chromatography on silica gel to separate and purify.



[Chemical formula A]

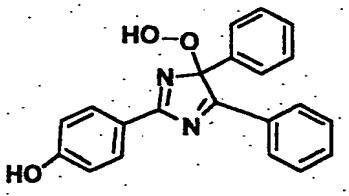
4-Hydroperoxy-2,4,5-triphenyl-4H-isoimidazole (E.H.White and M.J.C.Harding, Photochem. Photobiol., 1965, 4, 1129-1155): colorless powder; mp 108-110°C (dec.)(lit.[11], 110°C); IR(KBr) 1613(C=N) cm^{-1}; ^1H NMR(500 MHz, $CDCl_3$) δ7.22(t, J=7.8 Hz, 2H), 7.30-7.37(m, 4H), 7.48(dd, J=7.5, 5.5 Hz, 2H), 7.52(t, J=7.5 Hz, 2H), 7.59(t, J=7.5 Hz, 1H), 7.95(d, J= 7.5 Hz, 2H), 8.38(d, J= 7.5 Hz, 2H), 13.62(br s, 1H); ^{13}C NMR(67 MHz, DMSO-d_6) δ107.3(s), 124.5(d), 128.0(d), 128.6(d), 128.7(d), 128.9(d), 129.1(d), 129.5(d), 129.6(d), 131.3(s), 132.1(d), 132.8(d), 137.9(s), 169.6(s), 193.9(s); UV-vis λ_{max}(EtOH) 228(log ε 4.25), 281(4.32) nm; MS(FAB) m/z 329(M^++1); HRMS(FAB) Calcd for $C_{21}H_{17}N_2O_2$ 329.1265, Found 329.1290; Anal. Calcd for $C_{21}H_{17}N_2O_2$: C, 76.81; H, 4.91; N, 8.53. Found: C, 76.45; H, 4.94; N, 8.43.



[Chemical formula B]

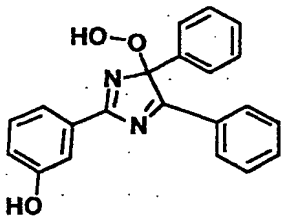
4,5-Bis(4-fluorophenyl)-4-hydroperoxy-2-(4-dimethylaminophenyl)-4H-isoimidazole (M. Kimura. H. Nishikawa, H. Kura., H. Lim, and E.H. White, CHEMISTRY LETTERS, 1993, 505-508): orange powder; mp 125-128°C (dec.); IR(KBr) 1603(C=N) cm^{-1}; ^1H NMR(500 MHz, $CDCl_3$) δ3.01(s, 6H), 6.42(d, J=8.9 Hz, 2H), 6.99(t, J=8.8 Hz, 2H), 7.17(t, J= 8.8 Hz, 2H), 7.44(dd, J=8.8, 5.3 Hz, 2H), 7.88(d, J= 8.9 Hz, 2H), 8.35(dd, J=8.8, 5.3 Hz, 2H), 12.82(br s, 1H); UV-vis λ_{max} (CH_2Cl_2) 229(log ε 4.1), 307(4.2), 402(4.1) nm; Anal. Calcd for $C_{23}H_{19}F_2N_3O_2$: C, 67.81; H, 4.70; N, 10.31. Found: C, 67.35; H, 4.66; N,

10.12.



[Chemical formula C]

4-Hydroperoxy-2-(4-hydroxyphenyl)-4,5-diphenyl-4H-imidazole: 288 mg of the raw material was irradiated with artificial daylight under bubbling oxygen for three hours to obtain it a pale yellow crystal (241 mg, 76%). mp 125-127°C (dec.); ^{1}H NMR(300MHz,DMSO-d_6) δ6.93(d, J=8.5Hz, 2H), 7.20-7.63(m, 8H), 8.08(d, J=7.3Hz, 2H), 8.17(d, J=8.5Hz, 2H), 10.2(s,1H), 12.2(br s, 1H); IR(KBr)ν_{max} 3396(O-H), 1607(C=N), 1510, 1437, 1319, 1278, 1170, 1087, 849, 754, 681cm^{-1}; UV-vis(DMSO) λ_{max} 295(log ε =4.29)nm; HRMS(FAB) Calcd for $C_{21}H_{17}N_2O_3$ 345.1239(M+H$^+$), Found 345.1252; Anal. Calcd for $C_{21}H_{16}N_2O_3 \cdot 1/2H_2O$: C, 71.38; H, 4.85; N, 7.93. Found: C,71.19; H, 4.88; N, 7.72.

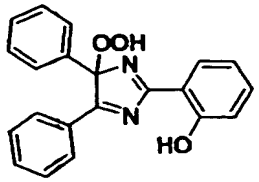


[Chemical formula D]

[0042] This compound was obtained as a pale yellow crystal (245 mg, 72%) by irradiating 399 mg of the raw material with artificial daylight under bubbling oxygen for three hours.

mp 111-113°C(dec.); ^{1}H NMR(300MHz, $CDCl_3$) δ6.87(ddd, J=7.8, 2.6, 1 Hz, 1H), 7.13(t, J=7.8 Hz, 1H), 7.29-7.63(m, 10H), 8.33(m, J=7.7Hz, 2H), 13.7(s,1H); IR(KBr)ν_{max} 3360(O-H), 1613(C=N), 1508, 1450, 1284, 780, 758, 743, 689cm^{-1}; UV-vis(CH_2Cl_2) λ_{max} 288(log ε =4.29)nm; HRMS(FAB) Calcd for $C_{21}H_{17}N_2O_3$ 345.1239(M+H$^+$), Found 345.1207; Anal. Calcd for $C_{21}H_{16}N_2O_3 \cdot 1/2H_2O$: C, 71.38; H, 4.85; N, 7.93. Found:

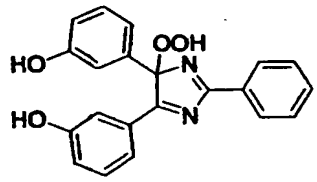
C,71.38; H, 4.87; N, 7.76.



[Chemical formula E]

4-Hydroperoxy-2-(2-hydroxyphenyl)-4,5-diphenyl-4H-isoimidazole

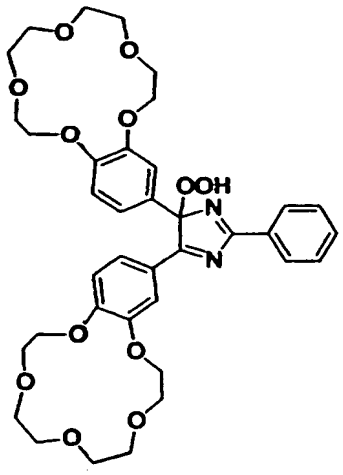
[0043] 2-(2-Hydroxyphenyl)-4,5-diphenyl imidazole (420 mg, 1.34 mmol) in CH_2Cl_2 (60 ml) and a catalytic amount of methylene blue in MeOH (1 ml) were irradiated with artificial daylight under an O_2 atmosphere at −78°C for seven hours. The reaction was monitored by TLC. Upon completion of the reaction, the sensitizer was removed by silica gel syringe column chromatography (CH_2Cl_2). The catalyst was concentrated under a reduced pressure and dried. The title compound was obtained as a purple crystal (346 mg, 75%).



[Chemical formula F]

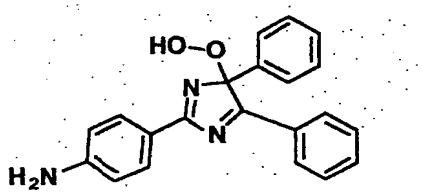
4-Hydroperoxy-4,5-bis(3-hydroxyphenyl)-2-phenyl-4H-isoimidazole

[0044] 4,5-Bis(3-hydroxyphenyl)-2-phenylimidazole (100 mg, 0.305 mmol) in CH_2Cl_2 and MeOH and an adduct polymer rose bengal (500 mg) were irradiated with artificial daylight under an O_2 atmosphere at −78°C for three hours. The reaction was monitored by TLC. Upon completion of the reaction, the sensitizer was removed by filtration. The catalyst was concentrated under a reduced pressure and the residues were dried. The title compound was obtained as a colorless crystal (95 mg, 86%).



[Chemical formula G]

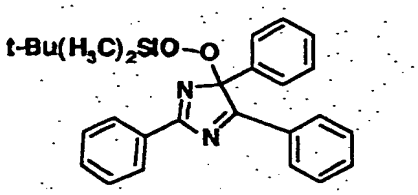
Bis(crown ether)iophineperoxide: yellow crystal; mp 99-101°C; ^{1}H NMR(500MHz, $CDCl_3$) δ13.61(bs), 8.00(m, 2H), 7.96(d, 1H, J=2.0Hz), 7.89(dd, 1H, J=2.0Hz, 8.5Hz), 7.40(bs,1H), 7.35-7.30(m, 1H), 7.24-7.20(m, 2H),6.91(d, 1H, J=8.5Hz), 6.74(dd, 1H, J=2.0Hz, 8.5Hz), 6.69(d, 1H, J=8.5Hz), 4.30-4.20 (m, 4H), 4.15-4.05(m, 4H), 3.97-3.92(m,4H), 3.86(t, 2H, J=4.5Hz), 3.82(t, 2H, J=4.5Hz), 3.80-3.75(m, 8H), 3.75-3.69(m, 8H)



[Chemical formula H]

2-(4-Aminophenyl)-4-hydroperoxy-4,5-diphenyl-4H-isoimidazole

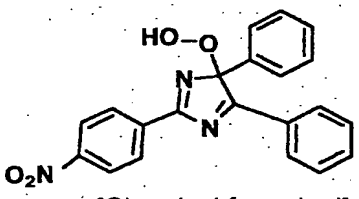
(E. Vedejs, and P. L. Fuchs, J. Org. Chem., 1971, 36,366-367.): yellow powder; mp 147-149°C (dec.); IR(KBr) 3376(N-H), 1603(C=N), 762, 692 cm^{-1}; ^{1}H NMR(500MHz, $CDCl_3$) δ4.02(br s, 2H), 6.54(d, J=9.0Hz, 2H), 7.29(m, 3H), 7.42-7.49(m, 4H), 7.55(t, J=7.5Hz, 1H), 7.93(d, J=9.0Hz, 2H), 8.32(d, J=7.5Hz, 2H); UV-visλ_{max} (EtOH) 209(log ε 4.33), 300(4.20), 376(3.95) nm; MS(FAB) m/z 344(M^++1)



[Chemical formula I]

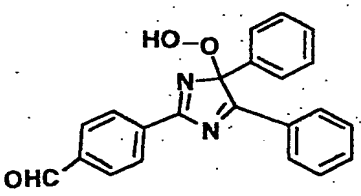
4-t-Butyldimethylsilylperoxy-2,4,5-triphenyl-4H-isoimidazole

colorless powder; mp 93.5-96.0 °C; IR(KBr) 2960(C-H), 1618(C=N), 886, 826(Si-O)cm^{-1}; ^1H NMR(500MHz, $CDCl_3$) δ0.145(s, 3H), 0.197(s, 3H), 0.843(s, 9H), 7.25-7.29(m, 3H), 7.30-7.35(m,2H), 7.43(t, J=8.0Hz, 2H), 7.49-7.58(m, 4H), 8.22(d, J=8.0Hz, 2H), 8.48(d, J=7.0Hz, 2Hz); UV-visλ_{max}(CH_2Cl_2) 232(log ε 4.22), 243(4.20), 279(4.30) nm; MS(FAB) m/z 443(M^++1); HRMS(FAB) Calcd for $C_{27}H_{31}N_2O_2Si$ 443.2155, Found 443.2139; Anal. Calcd for $C_{27}H_{30}N_2O_2Si \cdot 1/2H_2O$: C, 71.80; H, 6.92; N, 6.20. Found: C,72.06; H, 6.80; N, 6.17.



[Chemical formula J]

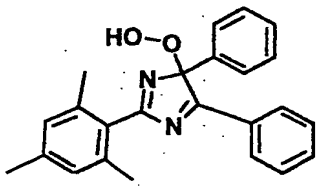
pale yellow powder; mp 148-159°C (dec.); IR(KBr) 1524(NO_2), 1350(NO_2) cm^{-1}; ^1H NMR(500MHz, $CDCl_3$) δ7.32-7.38(m, 3H), 7.43(dd, J=8.4, 2.0Hz, 2H), 7.55(t, J=8.1Hz, 2H), 7.65(t, J=8.1Hz, 1H), 8.08(d, J=9.2Hz, 2H), 8.18(d, J=9.2Hz, 2H), 8.37(d, J=8.1Hz, 2H), 12.9(s, 1H); UV-visλ_{max}(EtOH) (log ε) nm; MS(FAB) m/z 374(M^++1); Anal. Calcd for $C_{21}H_{15}N_3O_4 \cdot 1/4H_2O$: C, 66.75; H, 4.13; N, 11.12. Found: C,66.73; H, 4.00; N, 11.13;



[Chemical formula K]

2-(4-Formylphenyl)-4-hydroperoxy-4,5-diphenyl-4H-isoimidazole

[M. Kimura, M. Tsunenaga, T. Koyama, H. Iga, R. Aizawa, Y. Tachi, and Y. Naruta, ITE Letters on Batteries, New Technologies & Medicine, 1, C8 30-34(2002)]: pale yellow powder; mp 97.0-98.5 °C (dec.); IR(KBr) 1705(C=O), 1607(C=N), 835, 690 cm^{-1}; ^1H NMR(500MHz, $CDCl_3$) δ7.31-7.38(m, 3H), 7.45(m, 2H), 7.53(t, J=7.5Hz, 2H), 7.63(t, J=7.5Hz, 1H), 7.75(d, J=8.0Hz, 2H), 8.18(d, J=8.0Hz, 2H), 8.36(d, J=7.5Hz, 2H), 10.02(s, 1H), 12.69(br s, 1H); UV-visλ_{max} (EtOH) 281(log ε 4.48) nm; HRMS(FAB) Calcd for $C_{22}H_{17}N_2O_3$ 357.1239, Found 357.1216; Anal. Calcd for $C_{22}H_{16}N_2O_3$: C, 74.15; H, 4.53; N, 7.86. Found: C,74.29; H, 4.62; N, 9.44.



[Chemical formula L]

4-Hydroperoxy-2-(2',4',6'-trimethylphenyl)-4,5-diphenyl-4H-isoimidazole

[M. Kimura, M. Morioka, M. Tsunenaga, and Z-Z Hu, ITE Letters on Batteries, New Technologies & Medicine, 1, C25 418-421(2002)]: colorless powder; mp 157-158.5 °C (dec). (lit, 158-159.5 °C); IR(KBr) 2922(C-H), 1615(C=N) cm^{-1}; ^1H NMR(200MHz, $CDCl_3$) δ1.97(s, 6H), 2.31(s, 3H), 6.83(s, 2H), 7.35-7.57(m, 8H), 8.21(d, J=7.6Hz, 2H), 12.40(br s, 1H); UV-visλ_{max} (CH_2Cl_2) 229(log ε 4.07) 297(4.18) nm; Anal. Calcd for $C_{24}H_{22}N_2O_2$: C, 77.81; H, 5.99; N, 7.56. Found: C,77.64; H, 6.07; N, 7.57.

[0045] Test example 1

[Anticancer effect I]

Cytotoxicity was determined as shown below using MTT method proposed by Mosmann et al. (Mosmann, T.; Rapid colorimetric assay for cellular growth and survival: application proliferation and cytotoxicity assays. J. Immunol. Meth. 65: 55-63, 1983). An established cell line from human large intestine cancer was adjusted on a 10% FCS-containing RPMI 1640 culture solution to have 5 x 10^3

cells/100 ml, plated in a 96-well microplate, and incubated for 48 hours. Then, the resultant was added with 100 ml of a peroxide, and incubated under a 5% CO_2 condition at 37°C for 48 hours to determine cytotoxicity by MTT assay[1]. MTT assay: Upon completion of the incubation, 20 ml of MTT reagent (5 mg/ml in PBS) was added to each well, and then the formazan left on the bottom of the plate was added with 0.04N HCl to dissolve in isopropanol. OD was measured at a test wavelength and at a reference wavelength of 630 nm.

Survival rate in percentage was calculated by the following equation:

Survival rate = (OD test value/OD reference value) × 100 (%)

Results obtained for commercially available mitomycin C (MMC) are also shown in Table 1 for comparison.

[0046]

Table 1

Anticancer agent	Concentration of anticancer agent (μM/ml)	Survival rate of cancer cells %
Chemical formula A	1	100.1
	10	100.1
	100	78.3
Chemical formula B	1	100.3
	10	93.7
	100	37.0
Chemical formula C	1	91.9
	10	82.2
	100	11.1
Chemical formula D	1	93.3
	10	92.2
	100	71.2
Chemical formula E	1	-
	10	88.1
	100	11.8
Chemical formula F	1	-
	10	87.4
	100	42.2
Chemical formula G	1	-
	10	87.2
	100	48.2
Chemical formula H	1	100.0
	10	97.5
	100	87.4
Chemical formula I	1	97.2
	10	95.9
	100	60.6
MMC	1	65.0
	10	28.0
	100	14.5

[0047] As is evident from Table 1, Chemical formula C, Chemical formula E and the like of the present invention are comparable to the commercially available MMC in anticancer effect at a concentration of 100 μM/L. The results demonstrate that these peroxides are effective as anticancer agents.

[0048] Test example 2

[Measurement I]

Reaction heat and chemiluminescent efficiency relating to chemiluminescent reaction of the peroxides (Chemical formula A to Chemical formula L) were measured. Further, the amount of imidazole formed that indicates generation efficiency of singlet oxygen generated by this reaction was measured. Results thus obtained are summarized in Table 2.

[0049] Measurement of reaction heat: heat generated by above-mentioned compounds was measured by a differential thermal analyzer.

Heat measurement of a chemiluminescent system peroxide in the solid state was conducted using a differential thermal analyzer as follows: 2 to 3 mg of the peroxide was weighed, filled into an aluminum capsule, and heated gradually to 80 - 180°C by DSC-50 (Shimadzu Corporation) to measure the generated heat.

[0050] Measurement of relative light intensity of chemiluminescence: Reaction heat of the peroxides (Chemical formula A to Chemical formula L) in the solid state was measured and light intensity of the peroxides in the methanol solution mixed with 1N KOH methanol solution at a ratio of 10:1 was measured by PMA apparatus (Hamamatsu Photonics), and apparatus light intensity was determined while the luminescence of Chemical formula A was defined to be 1 for reference. Results are summarized in Table 2.

[0051] Formation of imidazole: the reaction solution was subjected to liquid chromatography to determine it: Developing phase Sephadex; Developer = Water : Ethanol (1:1). Results are summarized in Table 2.

[0052]

Table 2

Entry	Solution Reaction heat [a] / kcal/mol	Yield of imidazole [a] %	Solid reaction heat / kcal/mol	Solid reaction imidazole yield %	Relative amount of chemiluminescence [b]
Chemical formula A	53.8	13	18.7	45	1
Chemical formula B	66.3	~0	61.0	~0	160
Chemical formula C	---[c]	---[c]	53.6	---[c]	1.02
Chemical formula D	---[c]	---[c]	47.6	---[c]	0.232
Chemical formula H	46.0	3	35.3	~0	1.6
Chemical formula I	---[c]	---[c]	91.4	---[c]	0.58
Chemical formula J	48.7	49	52.3	50	0.60
Chemical formula K	22.4	58	15.0	55	~0
Chemical formula L	54.0	~0	50.5	~0	2.2

a) Reaction was started with 1N KOH/MeOH

b) Relative luminescence efficiency while Chemical formula A is defined to be 1 for reference.

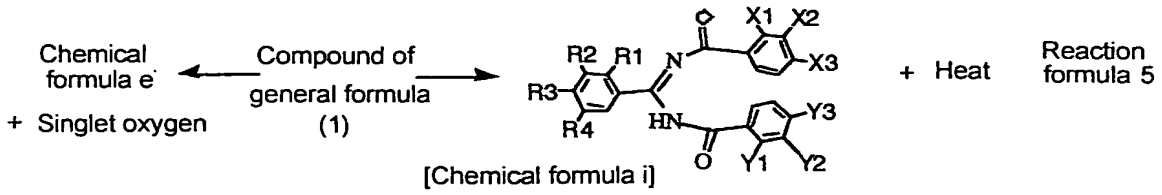
c) No measurement.

[0053] Test example 3

[Reaction example]

The peroxide represented by general formula (1) takes a chemiluminescent reaction and a reaction in an alcohol solvent in accordance with reaction formula (5) shown below. Singlet oxygen forms a pair with formation of imidazole (Chemical formula e), and generation of heat forms a pair with formation of amidine (Chemical formula i).

[0054] [Confirmation of singlet oxygen]



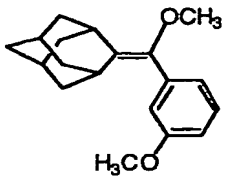
[Chemical formula i]

Constituents of a product given under a condition for chemiluminescent reaction were identified by HPLC. Measurement conditions: Column Intersil ODS-3 (46 mm × 150 mm); Solvent MeOH:H_2O = 7:3; rate 1.0 ml/min; reaction conditions: peroxide concentration: (5×10^{-3} M/$CHCl_3$) 1.0 ml, base concentration:0.5 M KOH/MeOH 0.10 ml, reaction time: Left to stand for 10 min. after mixing, and neutralized by acetic acid. Singlet oxygen was confirmed by an infrared spectrometer (Tohoku Electronic) and determined quantitatively with 1,3-diphenyl benzofuranbenzo. The corresponding imidazole [Chemical formula e] and singlet oxygen were formed in an equivalent amount. The amount of [Chemical formula e] formed can be determined to give the accurate amount of formed singlet oxygen. Although it is considered that the decomposition product is composed of [Chemical formula e] and the amidine [Chemical formula i], the amidine is easily hydrolyzed and hence could not be determined directly. It has been revealed that [Chemical formula A], [Chemical formula J] and [Chemical formula K] are particularly good singlet oxygen generators (Table 2).

[0055] Example 4

[Synthesis of dioxetanes]

[Chemical formula j] and [Chemical formula k] shown below were synthesized by the method of E.F. Ullman et al. (Unites States Patent 3,689,391 (1972)), and changed to the dioxetanes ([Chemical formula M] and [Chemical formula N]) of the present invention by reaction formula 6 or 7 shown below in accordance with peroxidation of an imidazole derivative.

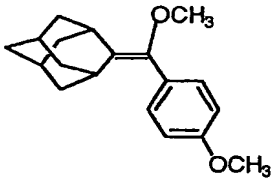


Methylene blue
Oxygen

Chemical formula M

Reaction formula 6

[Chemical formula j]

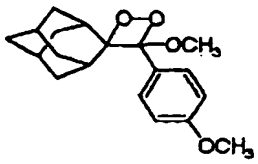


Methylene blue
Oxygen

Chemical formula N

Reaction formula 7

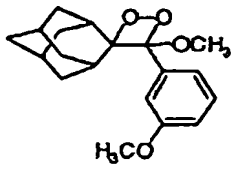
[Chemical formula k]



[Chemical formula M]

3-(2'-Spiroadamantane)-4-methoxy-4-(4"-methoxy)phenyl-1,2-dioxetane

^{1}H NMR(500MHz, $CDCl_3$) 0.97(d, J=12.0Hz, 1H), 1.23(d, J=13.3Hz, 1H), 1.45-1.81(m, 10H), 1.91(d, J=12.5Hz, 1H), 2.17(s, 1H), 3.02(s, 1H), 3.21(s, 3H), 3.84(s, 3H), 6.94(d, J=9.0Hz, 1H), 7.53(br s, 2H), 7.33 ppm (t, J=8.0Hz, 1H); IR(KBr) 2918, 1611, 1512, 1175 cm^{-1}



[Chemical formula N]

3-(2'-Spiroadamantane)-4-methoxy-4-(3"-methoxy)phenyl-1,2-dioxetane

^{1}H NMR(500MHz, $CDCl_3$) 1.03 (d, J=12.0Hz, 1H), 1.24(d, J=12.0Hz, 1H), 1.45-1.90(m, 10H), 2.12(s, 1H), 3.04(s, 1H), 3.23(s, 3H), 3.85(s, 3H), 6.94(d, J=8.0Hz, 1H), 7.18(br s, 2H), 7.33 ppm (t, J=8.0Hz, 1H); IR(KBr) 2920, 2860, 1586

cm^{-1}

[0056] Test example 4

[Measurement II]

Measurement of reaction heat: heat generated from above-mentioned compounds was measured by the differential thermal analyzer in accordance with above-mentioned [Measurement I]. Thermal measurement of a dioxetane compound in the solid state was carried out as follows: 2 to 3 mg of the dioxetane compound was weighed, filled into an aluminum capsule, and heated gradually to 80 - 180°C by DSC-50 (Shimadzu Corporation) to measure the generated heat. Results obtained are shown in Table 3.

[0057]

Table 3 Reaction heat of dioxetanes and survival rate of cancer cells

Entry	Solid reaction heat / kcal/mol	Survival rate of cells % (100 μM)[a]
Chemical formula M	68.8	55
Chemical formula N	66.8	67

a) Concentration of Chemical formula M and Chemical formula N for MTT assay

[0058] Test example 5

[Anticancer effect II]

Results of measurements by MTT assay shown in [Anticancer effect I] are shown in Table 3.

[0059] Example 5

Compounds of [Chemical formula O] and [Chemical formula P] shown below were synthesized in accordance with Example 1, Example 2 and Example 3. Specifically, terephthalaldehyde (0.340 g, 2.54 mmol), benzil of [Chemical formula g] (1.03 g, 4.90 mmol) and ammonium acetate (3.88 g, 50.4 mmol) were reacted in acetic acid

(60 mL) to obtain a crude product, which was then recrystallized from 1,4-dioxane or DMAc-H_2O to obtain compound of [Chemical formula 1] as colorless powder (1.18 g, 93%).

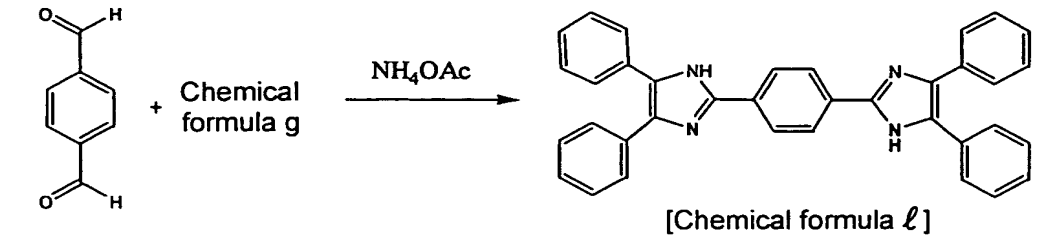
Results of analysis of compound of [Chemical formula 1] m.p. >300°C (IPE Letters, vol.3, p.30-34(2002), 410-412°C); ^{1}H NMR(300MHz, DMSO-d_6) δ 7.22-7.44(m, 12H), 7.45-7.59(m, 8H), 8.18(s, 4H), 12.8(br s, 1H); FT-IR(KBr) νmax 1605(C=N), 1489, 1444, 843, 766, 696 cm-1; UV(DMSO) λmax(log ε) 304 (sh) (4.32), 362 (4.69) nm; MS (m/z, FAB) 515(M+1); HRMS (FAB) Observed m/z 515.2238 ([M+H]$^+$), Calcd. for $C_{36}H_{27}N_4$ 515.2236; Elemental Analysis Calcd. for $C_{36}H_{26}N_4$: C 84.02, H 5.09, N 10.89, Found : C 83.33, H 5.11, N 10.80.

[0060] The compounds of [Chemical formula 1] (77.2 mg, 0.150 mmol) was added with methylene blue, and irradiated with artificial daylight while blowing oxygen for 13 hours to obtain the compound of [Chemical formula O] (43.4 mg, 50%) as pale yellow powder.

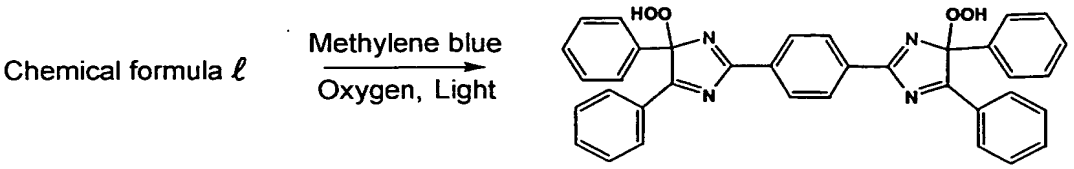
Results of analysis of compound of [Chemical formula O] m.p. 108°C (dec.); ^{1}H NMR(300MHz, DMSO-d_6) δ 7.25-7.70(m, J= 7.2Hz, 16H), 8.13(d, J= 7.2Hz, 4H), 8.54(m, 4H), 12.7(br s, 2H); FT-IR(KBr) νmax 1607(C=N), 1560.

[0061] The compound of [Chemical formula O] was subjected to t-butyldimethylsilylation to obtain the compound of [Chemical formula P] as colorless powder.

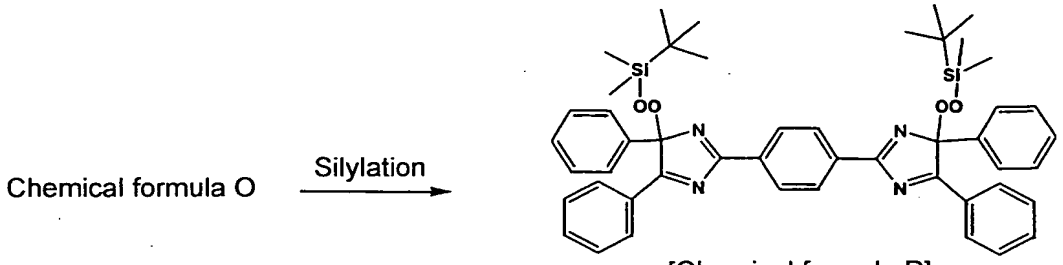
Results of analysis of compound of [Chemical formula P] m.p. 177-182°C (dec.); ^{1}H NMR(500MHz, $CDCl_3$) δ 0.17(s, 6H), 0.20(s, 6H), 0.84(s, 18H), 7.28-7.32(m, 6H), 7.33-7.37(m, 4H), 7.45(t, J= 7.5Hz, 4H), 7.53(t, J= 7.5Hz, 2H), 8.25(d, J= 7.5Hz, 4H), 8.61(s, 4H).



[Chemical formula ℓ]



[Chemical formula O]



[Chemical formula P]

[0062] [Chemical formula O] had a generation heat of 47.1 Kcal/mol, and [Chemical formula P] had a melting point of 177 - 182°C (decomposition), a generation heat of 147 Kcal/mol, and an imidazole yield of 41%.

[0063] Example 6

The compounds of [Chemical formula Q] and [Chemical formula R] shown below were synthesized in accordance with Example 1, Example 2 and Example 3. Isophthalaldehyde (0.275 g, 2.05 mmol), benzil of [Chemical formula g] (1.02 g, 4.85 mmol) and ammonium acetate (6.65 g, 86.2 mmol) were reacted in acetic acid (40 mL) to obtain a crude product, which was then recrystallized from ethyl acetate to obtain the compound of [Chemical formula m] (0.847 g,

69%) as a colorless needle.

[0064] Results of analysis of compound of [Chemical formula m]

m.p. 294-296°C; ^{1}H NMR(300MHz, DMSO-d_6) δ 7.20-7.47(m, 12H), 7.50-7.61(m, 9H), 8.07(d, J= 7.7Hz, 2H), 8.80(s, 1H), 12.8(br s, 2H); FT-IR(KBr) νmax 1603(C=N), 1485, 1456, 762, 694 cm^{-1}; UV(DMSO) λmax(log ε) 315(4.75) nm; MS (m/z, FAB) 515(M+1); HRMS (FAB) Observed m/z 515.2233 ($[M+H]^+$), Calcd. for $C_{36}H_{27}N_4$ 515.2236; Elemental Analysis Calcd. for $C_{36}H_{26}N_4 \cdot C_4H_8O_2$: C 79.71, H 5.69, N 9.30, Found : C 79.49, H 5.63, N 9.37.

[0065] The compound of [Chemical formula m] (216 mg, 0.358 mmol) was added with methylene blue, and irradiated with artificial daylight while blowing oxygen for seven hours to obtain the compound of [Chemical formula Q] (176 mg, 90%) as colorless powder.

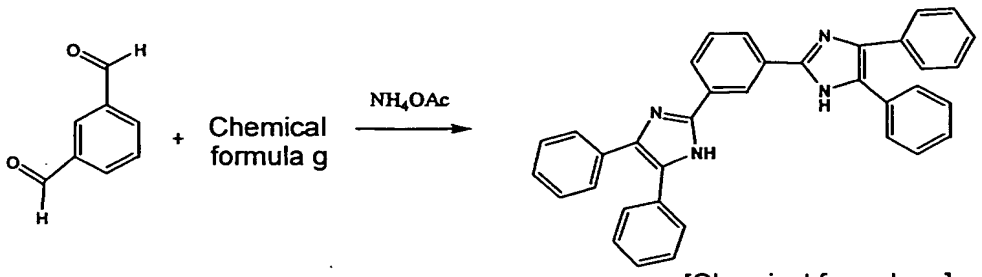
[0066] Results of analysis of compound of [Chemical formula Q]

m.p. 129-132°C (dec.); ^{1}H NMR(MHz, DMSO-d_6) δ 7.21-7.93(m, 17H), 8.14(d, J= 7.3Hz, 4H), 8.56(J= 8.7, 2Hz, 2H), 9.28(d, J= 2Hz, 1H); FT-IR(KBr) νmax 1618.

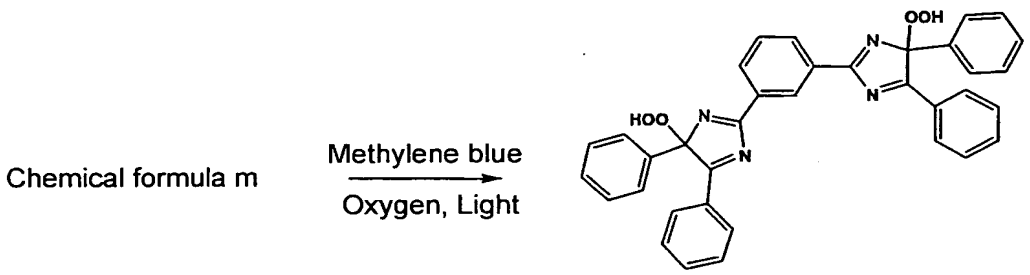
The compound of [Chemical formula Q] was subjected to t-butyldimethylsilylation to obtain the compound of [Chemical formula R].

[0067] Results of analysis of compound of [Chemical formula R]

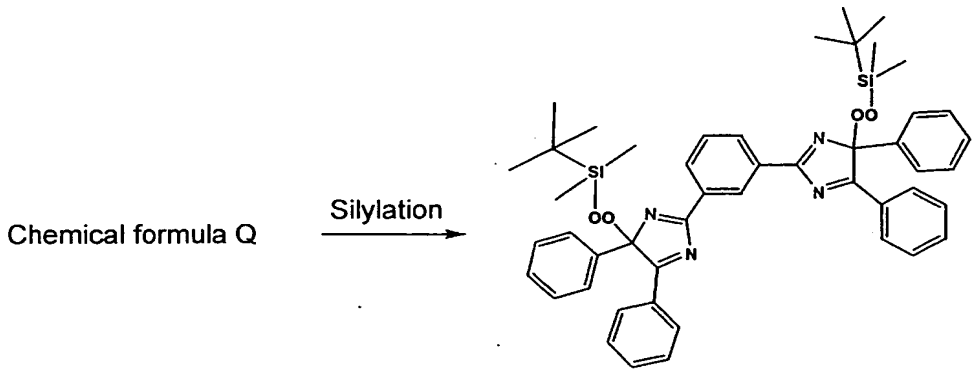
m.p. 52-61.5°C; ^{1}H NMR(200MHz, $CDCl_3$) δ 0.15(s, 6H), 0.18(s, 6H), 0.83(s, 18H), 7.20-7.70(m, 17H), 8.25(d, J= 8.0Hz, 4H), 8.60(m, 2H), 9.52(m, 1H).



[Chemical formula m]



[Chemical formula Q]



[Chemical formula R]

[0068] [Chemical formula Q] had a generation heat of 30.5 Kcal/mol and [Chemical formula R] had a generation heat of 143 Kcal/mol.

Industrial Applicability

[0069] The heat generator according to the present invention has a reaction heat of about 20 Kcal/mol to 90 Kcal/mol and/or a singlet oxygen yield of approximately

50%, and the pharmaceutical composition comprising the same hardly develops side effects and is hardly tolerated, imposes little burden on patients, and exhibits high anticancer activity.